

FORM PTO-1300
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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

0425-0837P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/851496

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/JP99/06336

November 12, 1999

November 18, 1998

TITLE OF INVENTION

DIFFUSION PROMOTING DEVICE FOR LOW FLOW VELOCITY GRADIENT HIGH PERFORMANCE LIQUID *

APPLICANT(S) FOR DO/EO/US

MURATA, Kaoru; MANO, Nariyasu; ASAKAWA, Naoki

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/29843
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is transmitted herewith.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98-International Search Report (PCT/ISA/210)
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
 - 1.) Seven (7) sheets of Formal Drawings
 - 2.) International Preliminary Examination Report (PCT/IPEA/409)

* CHROMOTOGRAPHY

U.S. APPLICATION NO. 09/831496		INTERNATIONAL APPLICATION NO. PCT/JP99/06336		ATTORNEY'S DOCKET NUMBER 0425-0837P	
21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4). \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). \$100.00 <input checked="" type="checkbox"/> ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
				\$ 860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 0	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	22 - 20 =	2	X \$18.00	\$ 36.00	
Independent Claims	3 - 3 =	0	X \$80.00	\$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes			+ \$270.00	\$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 1166.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$ 0	
SUBTOTAL =				\$ 1166.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0	
TOTAL NATIONAL FEE =				\$ 1166.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$ 40.00	
TOTAL FEES ENCLOSED =				\$ 1206.00	
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ 1206.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account. No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
Send all correspondence to: Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292 P.O. Box 747 Falls Church, VA 22040-0747 (703)205-8000					
Date: May 10, 2001			By <u>Raymond C. Stewart</u> Raymond C. Stewart, #21,066		

/ccq

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: MURATA, Kaoru et al. Conf.:
Int'l. Appl. No.: PCT/JP99/06336
Appl. No.: New Group:
Filed: May 10, 2001 Examiner:
For: DIFFUSION PROMOTING DEVICE FOR LOW FLOW
VELOCITY GRADIENT HIGH PERFORMANCE LIQUID
CHROMOTOGRAPHY

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

May 10, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/06336 which has an International filing date of November 12, 1999, which designated the United States of America and was not published in English.--

IN THE CLAIMS:

Please amend the claims as follows:

10. (Amended) A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), an injector (I), a switching valve (V) are connected in this order in one line; and a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1, a separation column (C) and a detector (D) are connected in another line, as shown in Fig. 8.

11. (Amended) A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line; a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1, a separation column (C) and a detector (D) are connected in another line; and a switching valve (V), a component concentration column (M) and a switching valve (V) are connected in a different line, as shown in Fig. 9.

REMARKS

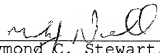
The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete improper multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  #6623
Raymond C. Stewart, #21,066

RCS/cqc
0425-0837P

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Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

VERSION WITH MARKINGS SHOWING CHANGES MADE

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

10. (Amended) A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), an injector (I), a switching valve (V) are connected in this order in one line; and a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1 [or 3], a separation column (C) and a detector (D) are connected in another line, as shown in Fig. 8.

11. (Amended) A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line; a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1 [or 3], a separation column (C) and a detector (D) are connected in another line; and a switching valve (V), a component concentration column (M) and a switching valve (V) are connected in a different line, as shown in Fig. 9.

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JC03 Rec'd POST/TC 10 MAY 2001

Description

Diffusion promoting device for low flow velocity gradient
high performance liquid chromatography

Technical Field

The present invention relates to a diffusion promoting device connected to just before a separation column and having a function for improving detection sensitivity in a low flow velocity gradient high performance liquid chromatography system, to a method for improving detection sensitivity of a target component by connecting the diffusion promoting device, and to an analytical method using the system.

Prior Art

High performance liquid chromatography has been frequently used for analyzing a trace amount of components in a sample. In recent years, high performance liquid chromatography is used in a system for simultaneously separating and identifying the component in a high sensitivity by combining with a mass spectrometer or a nuclear magnetic resonance spectrometer. For example, JP-A 3-175355 discloses an apparatus and a method for conversion of mobile phases in a high performance liquid chromatography-mass spectrometer system, and an apparatus

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for trapping a target components in a trapping column.

However, the permissible flow velocity of the mobile phase capable of flowing into the mass spectrometer is several tens microliters/min in the high performance liquid chromatography-mass spectrometer system from the view point of analytical accuracy of the mass spectrometer. A tube having a smaller inner diameter than conventional tubes is often used in the low flow velocity high performance liquid chromatography system as described above, in order to suppress diffusion of the target component in the tube of a liquid flow line. The inner diameter of the tube is generally determined by considering the inner diameter of a separation column and linear velocity in the tube.

When a tube having an inner diameter of 0.1 mm or less is used in the low flow velocity high performance liquid chromatography system, however, the tube may be blocked with fine particles to make an analysis by low flow velocity high performance liquid chromatography difficult.

It is known in the art, on the other hand, that an effect for concentrating the target component at the top portion of the separation column may be obtained when a gradient elution method is used in low flow velocity high performance liquid chromatography, that is in low flow velocity gradient high performance liquid chromatography. For example, JP-A 3-175355 discloses an example in which a tube having a larger inner diameter than usual, i.e. 0.8 mm,

and a length of 100 mm is connected at just before the separation column for efficiently obtaining the concentration effect. It reports that a gradient effect is obtained in the tube and peak resolution is improved by using such column as described above. However, since a tube as long as 100 mm is used in the method described above, it may be conjectured that improvement of resolution is not always realized depending on the kind of the separation column used, and on analysis conditions in high performance liquid chromatography.

Accordingly, development of an apparatus capable of enhancing analytical and quantitative sensitivity is urgently desired in the low flow velocity gradient high performance liquid chromatographic apparatus for analysis and assessment of a trace amount of biological components, and impurities in environmental samples and pharmaceuticals.

Brief Description of the Drawings

Fig. 1 illustrates a diffusion promoting device comprising a solvent inlet tube (1) and outlet tube (2), a part (3) of the solvent inlet tube or outlet tube having a larger inner diameter than the other portion of the tube.

Fig. 2 illustrates a diffusion promoting device comprising a solvent inlet tube (1) and outlet tube (2), a part (3) of the solvent inlet tube or outlet tube having a larger inner diameter than the other portion of the tube,

and a frit (4) being inserted into the portion having a larger inner diameter.

Fig. 3 illustrates a diffusion promoting device comprising a solvent inlet tube (1) and outlet tube (2), a part (3) of the solvent inlet tube or outlet tube having a larger inner diameter than the other portion of the tube, and the portion having a larger inner diameter being tapered.

Fig. 4 illustrates a diffusion promoting device comprising a solvent inlet tube (1) and outlet tube (2), a part (3) of the solvent inlet tube or outlet tube having a larger inner diameter than the other portion of the tube, the portion having a larger diameter being tapered, and a flit (4) being inserted in front of the tapered portion.

Fig. 5 illustrates a diffusion promoting device in which the solvent inlet tube (1) and outlet tube (2) are connected forming an acute angle.

Fig. 6 illustrates a diffusion promoting device in which the solvent inlet tube (1) and outlet tube (2) are connected forming a rectangle.

Fig. 7 illustrates a diffusion promoting device in which the solvent inlet tube (1) and outlet tube (2) are connected forming an obtuse angle.

Fig. 8 illustrates a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device is connected at just before a separation

Fig. 9 illustrates a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device is connected at just before a separation column.

Fig. 10 shows a chromatogram obtained by using a low flow velocity gradient high performance chromatographic apparatus in which no diffusion promoting device is connected.

Fig. 11 shows a chromatogram obtained by using a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device according to the present invention shown in Fig. 1 is connected.

Fig. 12 shows a chromatogram obtained by using a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device according to the present invention shown in Fig. 2 is connected.

Fig. 13 shows a chromatogram obtained by using a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device according to the present invention shown in Fig. 5 is connected.

Fig. 14 shows a chromatogram obtained by using a low flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device according to the present invention shown in Fig. 6 is connected.

Fig. 15 shows a chromatogram obtained by using a low

flow velocity gradient high performance chromatographic apparatus in which a diffusion promoting device according to the present invention shown in Fig. 7 is connected.

Disclosure of Invention

The inventors of the present invention has found, through intensive studies considering the situations as described above, that the desired objects can be attained by the construction as described below.

The present invention provides a diffusion promoting device provided just before a separation column and having a function for improving detection sensitivity, in a low flow velocity high performance liquid chromatographic apparatus.

The present invention also provides a method for improving detection sensitivity of a target component for use in a low flow velocity gradient high performance liquid chromatographic apparatus, wherein a diffusion promoting device is provide just before a separation column.

The present invention further provides a method for analyzing a trace amount of the target component for use in a low flow velocity high performance liquid chromatographic apparatus by concentration, diffusion and separation of the target component to be analyzed in the sample.

The diffusion promoting device comprises a solvent inlet tube and outlet tube, which satisfies 1) a part of

either the solvent inlet tube or the solvent outlet tube has a larger inner diameter than the other does or 2) the solvent inlet tube and outlet tube are connected with a given angle with each other, or both 1) and 2).

The diffusion promoting device is connected between a component concentration column and the separation column through a tube in the low flow rate gradient high performance liquid chromatography system in which the component concentration column is connected at just before the separation column. The diffusion promoting device has a function by which a sample band containing the target component eluted from the component concentration column is diffused by changing the solvent flow passageway in the device to form a homogeneous solution. Since this homogeneous solution is introduced into the separation column, a concentrating effect of the component at the top portion of the separation column in gradient elution is efficiently exerted. Consequently, theoretical plates of the separation column to be used for assessment of the target component is increased while improving the peak shape of the component, thereby enabling sensitivity of qualitative detection of the target component to be improved.

A tubing with an inner diameter of 0.1 mm or less is usually used in low flow velocity gradient high performance liquid chromatography system. However, it is possible to

improve detection sensitivity of the target component in the low flow velocity gradient high performance liquid chromatography system in which the diffusion promoting device according to the present invention is connected, by, for example, making the inner diameter of a part of the solvent inlet tube and outlet tube in the device to be larger (an inner diameter of 0.13 mm or 0.25 mm) than the other part, and by using a tube with an inner diameter of 0.13 mm or less for the other portion of the line. It is also possible to prevent the inside of the tube connected at just before the separation column and the inside of the separation column from being clogged, by elongating the portion having a large inner diameter between the solvent inlet and outlet tubes in the diffusion promoting device.

The given angle formed between the solvent inlet tube and outlet tube in the diffusion promoting device may be any one of an acute angle, a rectangle and an obtuse angle. Otherwise, a frit membrane may be inserted into the diffusion promoting device. While examples of the frit (membrane) include a sintered filter, a ceramic, a metal mesh or a cellulose membrane, they are not necessarily restricted thereto.

In particular, the diffusion promoting device comprises a solvent inlet tube and outlet tube, which satisfies 1) a part of either the solvent inlet tube or the solvent outlet tube has a larger inner diameter than the

other portion or 2) the solvent inlet tube and outlet tube are connected with a given angle with each other, or both 1) and 2), and wherein a frit (membrane) is inserted in the inlet tube and/or outlet tube, both objects of promoting diffusion of the target component and efficiently preventing clogging of the separation column with fine particles in the separation column can be simultaneously attained.

The diffusion promoting device may display the functions for improving detection sensitivity and/or for preventing clogging in the device, so long as the device is assembled into low flow velocity high performance liquid chromatographic apparatus. The low flow velocity high performance liquid chromatographic apparatus includes, for example, a gradient micro high performance chromatographic apparatus having a micro column with an inner diameter of 0.5 to 1 mm and being used at a flow velocity of several tens microliters per minutes, a gradient semi-micro high performance liquid chromatographic apparatus having a semi-micro column with an inner diameter of 1 to 2.5 mm and being used at a flow velocity of 50 to 250 μ l/min, or a gradient capillary high performance liquid chromatographic apparatus having a capillary column with an inner diameter of 0.5 mm or less and being used at a flow velocity of several microliters per minutes.

The diffusion promoting device is preferably provided

at just before the separation column, more preferably between the component concentration column and separation column, in the low flow velocity liquid chromatographic apparatus.

While one example of the low flow velocity liquid chromatographic apparatus in which the diffusion promoting device according to the present invention is connected at just before the separation column is described in detail hereinafter, the present invention is by no means restricted thereto.

As shown in Fig. 8, the present invention provides a low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), an injector (I), a switching valve (V), a component concentration column (M), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line, and a solvent pump (P2), a switching valve (V), a diffusion promoting device (DU), a separation column (C) and a detector (D) are connected in another line.

The present invention also provides a method for analyzing a trace amount of a component in a sample with high detection sensitivity for use in the low flow velocity gradient high performance liquid chromatographic apparatus, wherein the target component is trapped in the component concentration column (M) by means of a mobile phase discharged from the solvent pump (P1), the switching valve

(V) is turned to discharge another mobile phase using a solvent pump (P2), and the target component is eluted from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU).

As shown in Fig. 9, the present invention provides a low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line, and a solvent pump (P2), a switching valve (V), a diffusion promoting device (DU), a separation column (C) and a detector (D) are connected in another line, and a switching valve (V), a component concentration column (M) and a switching valve (V) are connected in a different line. The present invention also provides a method for analyzing a trace amount of a component in a sample with high detection sensitivity for use in the low flow velocity gradient high performance liquid chromatographic apparatus, wherein the target component is injected into the component concentration column (M) while filling a solvent in the solvent mixer (MC) by means of the solvent pump (P1), the switching valve is turned to discharge a mobile phase by means of a solvent pump (P2), and the target component is eluted from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU).

Although the diffusion promoting device according to the present invention manufactured by machining and combining the solvent inlet tube and solvent outlet tube may be used as an exposed device, it may be housed in a small size vessel. The materials for the solvent inlet and outlet tubes, and for the vessel are not particularly restricted.

While the small size vessel easy for use may be a linear union, a triangular union or a T-shaped union, the solvent inlet tube and outlet tube are desirably tightly screwed using, for example, ferrules in order to prevent the mobile phase from leaking.

While examples of the diffusion promoting device are illustrated in Figs. 1 to 7, the device is by no means restricted thereto.

While the solvent inlet tube (1) is connected to the component concentration column, and the solvent outlet tube (2) is connected to the separation column in the diffusion promoting devices shown in Figs. 1 to 7, they may be connected in a reverse order with each other. For example, a good diffusion promoting effect may be obtained when the tube (1) is connected to the separation column and the tube (2) is connected to the component concentration column.

Fig. 1 shows an example using the liner union in which the inner diameter of a part (3) of the solvent inlet tube (1) and outlet tube (2) is larger than the other

portion, and Fig. 2 shows an example in which a frit (4) is inserted into the part (3) having a larger inner diameter than the other portion in the diffusion promoting device.

Fig. 3 shows the diffusion promoting device with the same mechanism as that in Fig. 1, wherein a part (3) of the solvent inlet tube (1) and outlet tube (2) also has a larger inner diameter than the other portions. Fig. 4 shows the diffusion promoting device in which the flit (4) is inserted into the portion (3) having a larger inner diameter than the other portion in the device. In a preferred example as shown in Figs. 3 and 4, the passageway of the mobile phase in the diffusion promoting device is tapered from the inlet tube (1) toward the outlet tube (2), or from the outlet tube (2) toward the inlet tube (1), in order to facilitate diffusion of the sample in the diffusion device.

In the diffusion promoting device shown in Figs 5 to 7, the solvent inlet tube (1) is connected to the solvent outlet tube (2) with a given angle. For example, the triangular union is used in the device shown in Figs. 5 and 7, and the T-shaped union is used in the device shown in Fig. 6. The angles formed between the inlet tube (1) and outlet tube (2) is an acute angle, a rectangle and an obtuse angle in the devices shown in Figs. 5, 6 and 7, respectively. These angles between the tubes functions to change the solvent passageway to thereby allow the sample

band to diffuse. As shown in Fig. 5, a frit (4) such as a line filter may be inserted into a part of the tube in the device.

A system being improved in detection sensitivity and suitable for high speed, high sensitivity analysis of trace components may be constructed by integrating the diffusion promoting device into the low flow velocity gradient high performance liquid chromatography system configured as described below.

The system according to the present invention will be described in detail with reference to Fig. 8.

Fig. 8 illustrate the low flow velocity gradient high performance liquid chromatography system in which a solvent pump (P1), an injector (I), a switching valve (V), a component concentration column (M) comprising a diffusion membrane and an absorption membrane, a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line, and a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU), a separation column (C) and a detector (D) are connected in another line. The concentration and separation method in this low flow velocity gradient high performance liquid chromatography method is as follows.

(A) A mobile phase for component concentration is discharged from the solvent pump (P1), the sample solution is injected into the injector (I), and the sample is

(B) Then, the mobile phase for sample separation discharged from the pump (P2) is eluted by turning the valve (V) through the solvent mixer (MC), sample concentration column (M), diffusion promoting device (DU), separation column (C) and detector (D). The mobile phase for sample separation serves as a mobile phase for eluting the sample components from the sample component concentration column (M) and separating the sample components through separation column (C). A solvent having smaller polarity than the mobile phase such as methanol and acetonitrile is used for component concentration when the component concentration column (M) is hydrophobic. The mobile phase for component concentration is mixed with the mobile phase for sample separation by means of the solvent mixer (MC), and the mixed mobile phase is pumped into the

component concentration column (M) with a gradient in the mixing ratio between the two mobile phases to elute the trapped target component in the sample. A homogeneous solution is formed by allowing the sample band containing the target component to diffuse by changing the solvent passageway in the diffusion promoting device (DU). Since this homogeneous solution is introduced into the separation column, theoretical plates of the separation column to be used for assessment of the target component increases and the shape of the corresponding peak is improved in the low flow velocity gradient high performance liquid chromatography system.

In summary, the system according to the present invention is featured in remarkable improvement of quantitative detection sensitivity of the target component in the separation column.

The pump as used herein is a solvent pump for the high performance liquid chromatography system, and preferably a solvent pump for the low flow velocity high performance liquid chromatography system. The valve is, for example a ten-way valve or a six-way valve for the high performance liquid chromatography system. The injector is a device for injecting the sample solution into the high performance liquid chromatography system. The separation column is a column for separating the target component in the sample, and a so-called normal phase column and

reversed phase column are appropriately used depending on purposes. These devices are commercially available.

Another system for low flow velocity gradient high performance liquid chromatography according to the present invention is described with reference to Fig. 9. Fig. 9 illustrates the low flow velocity gradient high performance liquid chromatography system in which the solvent pump (P1), switching valve (V), solvent mixer (MC) and the switching valve (V) are sequentially connected in one line; the solvent pump (P2), the switching valve (V), diffusion promoting device (DU), separation column (C) and detector are separately connected; and the switching valve (V), component concentration column (M) and switching column are further connected in a different line.

The concentration and separation method of the components in the system shown in Fig. 9 is as follows.

(A) The mobile phase (1) is discharged from the solvent pump (1) to fill the solvent mixer (MC). The sample solution is injected into the component concentration column connected to the switching valve (V) through an injection port of the switching valve and, after allowing the target component in the sample to be trapped with the component concentration column, the column is washed with an appropriate solvent so that the target component is not eluted from the membrane.

(B) Then, the mobile phase for sample separation is

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pumped from the pump (P2) into the solvent mixer (MC), component concentration column, separation column (C) and detector (D) by turning the switching valve (V). The mobile phase 1 and the mobile phase for sample separation are mixed in the solvent mixer (MC), and the mixed solvent is pumped into the component concentration column with a gradient in the mixing ratio between the two solvents. The target component in the trapped sample is eluted, and the sample band containing the target component is allowed to diffuse in the diffusion promoting device (DU) by switching the solvent flow passageway to form a homogeneous solution. The solution flows through the separation column (C) to separate the target component. The direction of the solvent flow passageway of the mobile phase for sample separation flowing through the component concentration column is opposite to injection direction of the sample solution. The sample solution may be manually injected into the component concentration column in the system shown in Fig. 9, thereby enabling a large scale processing and high speed concentration of the sample solution.

According to the present invention, detection sensitivity of the target component can be improved by connecting the diffusion promoting device at just before the separation column in the low flow velocity gradient high performance liquid chromatographic apparatus.

Example

Effect of configuration of the diffusion promoting device on the peak height and theoretical plate of the target component in the low velocity gradient high performance liquid chromatographic apparatus

Peak shapes in the chromatogram were assessed after injecting sample components into a low velocity gradient high performance liquid chromatographic apparatus as shown in Fig. 8, wherein either a linear union as shown in Fig. 1, a linear union having a frit as shown in Fig. 2, or a three-way union having a different angles between the solvent inlet tube and outlet tube as shown in Fig. 5, 6 or 7 was connected as the diffusion promoting device (DU). Peaks obtained by using a low velocity gradient high performance liquid chromatographic apparatus in which no diffusion promoting device was connected were also assessed as references.

The sample components used were n-propyl benzoate (A), benzyl benzoate (B), n-butyl benzoate (C) and n-hexyl benzoate (D), and each sample was dissolved in an aqueous solution containing 10% of acetonitrile in a concentration of 10 $\mu\text{g/ml}$. The injection volume of the sample solution was 10 μl .

Figs. 10 to 15 shows the chromatograms when the respective diffusion promoting devices having different shapes with each other are connected in the low velocity

gradient liquid chromatographic apparatus. The chromatograms of the sample components A to D are shown in Fig. 10 when no diffusion promoting device is connected, in Fig. 11 when the diffusion promoting device shown in Fig. 1 is connected, in Fig. 12 when the diffusion promoting device shown in Fig. 2 is connected, in Fig. 13 when the diffusion promoting device shown in Fig. 5 is connected, in Fig. 14 when the diffusion promoting device shown in Fig. 6 is connected and in Fig. 15 when the diffusion promoting device shown in Fig. 7 is connected, respectively, to the low velocity gradient liquid chromatographic apparatus.

The peak of each sample component was sharpened in the chromatograms (Figs. 11 to 15) obtained by using the low velocity gradient high performance liquid chromatographic apparatus in which respective diffusion promoting devices are connected, as compared with the chromatogram (Fig. 10) obtained by using the low velocity gradient high performance liquid chromatographic apparatus in which no diffusion promoting device is connected. In particular, increase of the peak heights of the sample components (B) and (C) was remarkable.

The peak heights and theoretical plates were calculated with respect to the component (C) as assessment items of the peaks in the chromatograms from the chromatograms shown in Figs. 10 to 15, in order to investigate the effect of each configuration of the

diffusion promoting device on both factors.

In general, peak resolution becomes insufficient in the assay of a mixture when the peak width of the target component is broadened and overlapped with the adjoining peaks. Accordingly, an experimental condition that prevent the peaks from being broadened should be determined, and a theoretical plate (N) is usually used as a measure of the peak broadening. The theoretical plate is expressed as $(4VR/W)^2$, wherein VR is the retention time of the target component and W is the peak width of the target component.

The experimental results were summarized in Table 1.

Table 1

Configuration of the diffusion promoting device	Peak height	Theoretical plate (N)
No device	83640	38612
Linear union (no frit)	136915	-
Linear union (with frit)	128862	71728
Flow passageways crossing at an acute angle	125570	-
Flow passageways crossing at a rect angle	108062	66915
Flow passageways crossing at an obtuse angle	122534	-

The peak height of the target component and theoretical plate of the separation column were clearly improved by using the low flow velocity gradient high performance liquid chromatographic apparatus in which the diffusion promoting device was connected, as compared with those obtained by using the low flow velocity gradient high

performance liquid chromatographic apparatus in which no diffusion promoting device was connected. Few differences in both factors ascribed to the difference of the configurations of the diffusion promoting device were observed.

However, as shown in Figs. 11, 13 and 15, calculation of the theoretical plate was impossible when the peak is so sharpened that its tip reached beyond the upper limit on the recording paper as a result of using the diffusion promoting device.

Further, clogging of neither the tube placed at just before the separation column nor the separation column itself was observed in the low flow velocity high performance gradient liquid chromatographic apparatus in which the diffusion promoting device was connected.

As hitherto described in detail, the diffusion promoting device according to the present invention that is connected at just before the separation column clearly has a function for improving detection sensitivity in the low flow velocity high performance gradient liquid chromatographic apparatus.

The experimental data in Table 1 and chromatograms in Figs. 10 to 15 were obtained by the following experimental conditions.

Detector: UV absorption photometer (measuring wavelength: 254 nm)

Separation column: Inertsil ODS-2 (0.7 mm I.D. x 150 mm)

Mobile phase for concentration: aqueous solution containing

0.1% ammonium acetate

Mobile phase for sample separation: mixed solution of

acetonitrile containing 0.1% ammonium acetate and ethanol

(500:500)

Flow velocity

Mobile phase for for sample concentration: 1.0 ml/min

Mobile phase for sample separation: 0.025 ml/min

Claims

1. In a low flow velocity gradient high performance liquid chromatographic apparatus, a diffusion promoting device provided just before a separation column and having a function for improving detection sensitivity.

2. A method for improving a detection sensitivity of a target component, which comprises providing the diffusion promoting device just before a separation column in a low flow velocity gradient high performance liquid chromatographic apparatus.

3. The diffusion promoting device according to Claim 1, which comprises a solvent inlet tube and a solvent outlet tube and satisfies the requirement 1) a part of either the solvent inlet tube or the solvent outlet tube has a larger inner diameter than the other portion; and 2) the solvent inlet tube and the solvent outlet tube are connected with a given angle with each other, or satisfies the both requirements 1) and 2).

4. The diffusion promoting device according to Claim 3, wherein the given angle formed between the solvent inlet tube and the solvent outlet tube is an acute angle, a rectangle or an obtuse angle.

5. The diffusion promoting device according to Claim 1 or 3, wherein a frit is inserted in the solvent inlet tube and/or outlet tube.

6. The diffusion promoting device according to Claim 5, wherein the frit is a sintered filter, a ceramic, a metal mesh or a cellulose fiber.

7. The diffusion promoting device according to Claim 1, wherein the low flow velocity gradient high performance liquid chromatographic apparatus is a gradient micro high performance liquid chromatographic apparatus, a gradient semimicro high performance liquid chromatographic apparatus, or a gradient capillary high performance liquid chromatographic apparatus.

8. A low flow velocity high performance liquid chromatographic apparatus comprising the diffusion promoting device according to Claim 1 or 3 at just before the separation column.

9. A low flow velocity high performance liquid chromatographic apparatus in which the diffusion promoting device according to Claim 1 or 3 is connected between the component concentration column and the separation column.

10. A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), an injector (I), a switching valve (V), a component concentration column (M), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line; and a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1 or 3, a separation column (C) and a detector (D)

are connected in another line, as shown in Fig. 8.

11. A low flow velocity high performance liquid chromatographic apparatus in which a solvent pump (P1), a switching valve (V), a solvent mixer (MC) and a switching valve (V) are connected in this order in one line; a solvent pump (P2), a switching valve (V), the diffusion promoting device (DU) according to Claim 1 or 3, a separation column (C) and a detector (D) are connected in another line; and a switching valve (V), a component concentration column (M) and a switching valve (V) are connected in a different line, as shown in Fig. 9.

12. A method for analyzing a trace amount of a component in a sample with improved detection sensitivity for use in the low flow velocity gradient high performance liquid chromatographic apparatus according to Claim 10, which comprises trapping the target component in the component concentration column (M) by means of a mobile phase discharged from the solvent pump (P1); discharging a different mobile phase from the solvent pump (P2) by turning the switching valve; and eluting the target component from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU) according to Claim 1 or 3.

13. A method for analyzing a trace amount of a component in a sample with improved detection sensitivity for use in the low flow velocity gradient high performance

liquid chromatographic apparatus according to Claim 11, which comprises injecting the target component into the component concentration column (M) while filling a solvent in the solvent mixer (MC) by means of the solvent pump (P1); discharging a mobile phase from the pump (P2) by turning the switching valve; and eluting the target component from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU) according to Claim 1 or 3.

14. A method for analyzing a trace amount of a target component for use in a low flow velocity gradient high performance liquid chromatography by concentration, diffusion and separation of the target component in a sample.

Abstract

The present invention provides a device for improving detection sensitivity and a method for improving the detection sensitivity for use in a low flow velocity high performance liquid chromatographic apparatus. That is, the present invention is a diffusion promoting device connected at just before a separation column and having a function for improving detection sensitivity for use in a low flow velocity high performance liquid chromatographic apparatus.

0031496-051004

Fig. 1

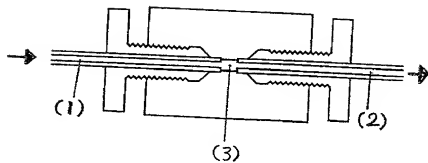


Fig. 2

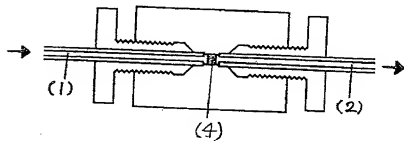


Fig. 3

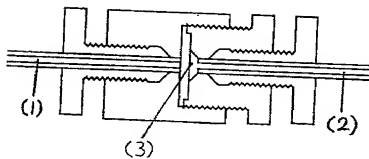


Fig. 4

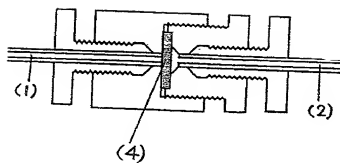


Fig. 5

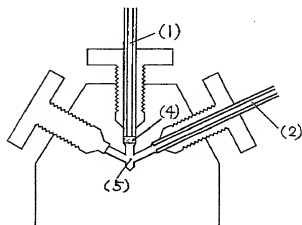


Fig. 6

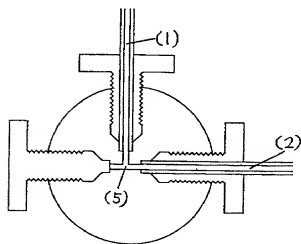


Fig. 7

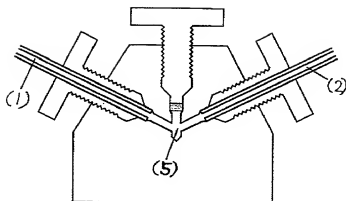


Fig. 8

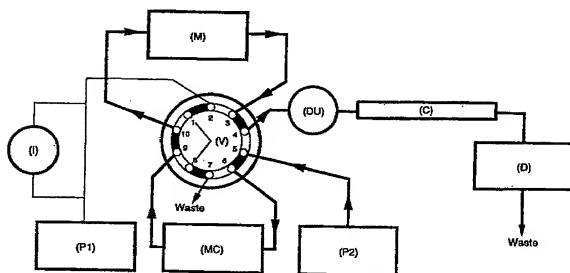


Fig. 9

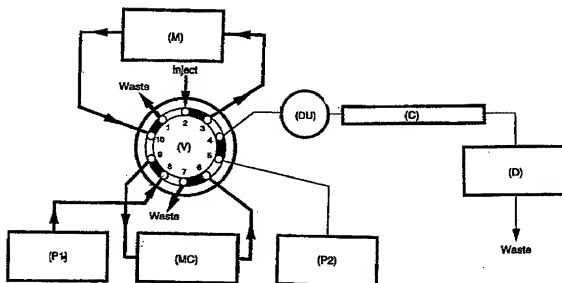


Fig. 10

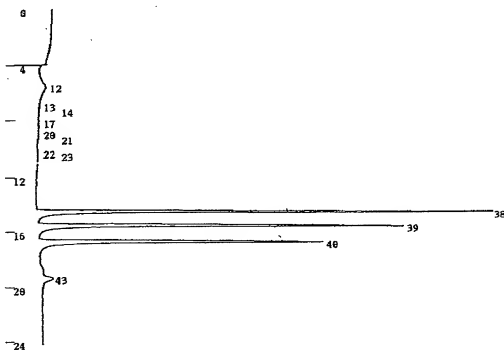


Fig. 11

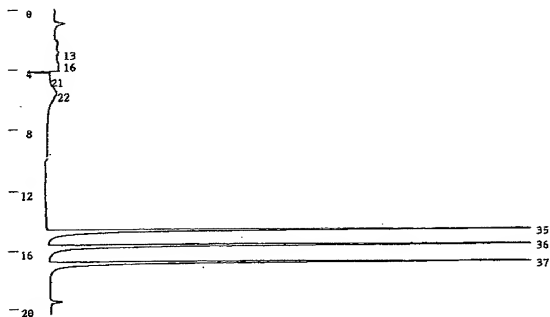


Fig. 12



Fig. 13

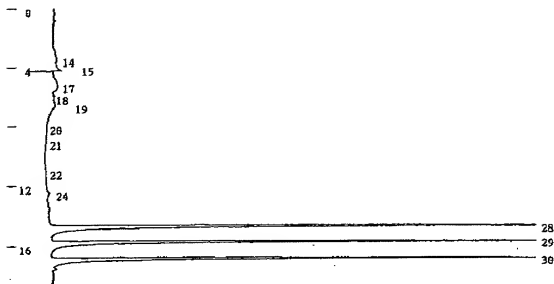


Fig. 14

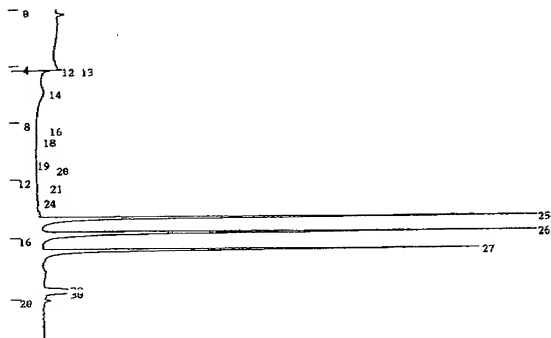


Fig. 15



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Diffusion promoting device for low flow velocity gradient
high performance liquid chromatography

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Specification
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the specification of which is attached hereto. If not attached hereto,

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United States Application Number _____; and /or

the specification was filed on November 12, 1999 as PCT
International Application Number PCT/JP99/06336; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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Insert Priority
Information:
(if appropriate)

Prior Foreign Application(s)
10-328465

Japan

Nov. 18, 1998

Priority Claimed

(Number)

(Country)

(Month/Day/Year Filed)

☒☐

Yes

No

(Number)

(Country)

(Month/Day/Year Filed)

☐☐

Yes

No

(Number)

(Country)

(Month/Day/Year Filed)

☐☐

Yes

No

(Number)

(Country)

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Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Insert Prior U.S.
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(if any)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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Full Name of Third
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Residence (City, State & Country)		CITIZENSHIP	
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